

## **Integrated Investigation on the Production and Fate of Organo-Cr(III) Complexes from Microbial Reduction of Chromate**

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**RESULTS TO DATE:** Our objective is to investigate the complexity of chromium biogeochemistry. Our results clearly support more complexity. In short, the chromium cycle is not as simple as the conversion between Cr(III) and Cr(VI) in inorganic forms. We have obtained more evidence to prove the formation of soluble organo-Cr(III) complexes from microbial reduction of Cr(VI). The complexes are relatively stable due to the slow ligand exchange of Cr(III). However, some microorganisms can consume the organic ligands and release Cr(III), which then precipitates. Efforts are being made to characterize the organo-Cr(III) complexes and investigate their behavior in soil. Progress and efforts are summarized for each task. Task 1. Production of soluble organo-Cr(III) complexes by selected microorganisms. A total of eight organisms were screened for production of soluble organo-Cr(III) complexes by culturing in both growth and non growth media containing 4 mg/L of Cr(VI); three were Gram positive and five were Gram negative. The Gram-positive bacteria were *Cellulomonas* sp. ES 6, *Rhodococcus* sp., and *Leafsonia* sp., while *Shewanella oneidensis* MR 1, *Desulfovibrio desulfuricans* G20, *D. vulgaris* Hildenborough, *Pseudomonas putida* MK 1 and *Ps. aeruginosa* PAO 1 were Gram negative. Purifications of the soluble organo-Cr(III) complexes produced by *Cellulomonas* sp. ES 6, *Shewanella oneidensis* MR 1, *Rhodococcus* sp., and *D. vulgaris* Hildenborough were carried out. The culture supernatants were lyophilized and extracted first with methanol followed by water. The extracts were then analyzed for soluble Cr. The majority of the Cr(III) was present in the water-soluble fraction for all of the bacteria tested (data not shown), revealing a general phenomenon of soluble Cr(III) production. *Cellulomonas* sp. ES6 produced the highest amount of soluble Cr(III) (364 ppm) and *D. vulgaris* Hildenborough produced the least (143 ppm). Seventy eight percent of the soluble Cr(III) produced by *Shewanella oneidensis* MR 1 was water soluble, while 45% was water soluble for the *Cellulomonas* sp. ES6. The water-soluble fractions were further purified by anion exchange chromatography. All soluble Cr(III) was bound to the anion exchange column. The bound organo-Cr(III) was eluted by gradient elution, (0.25M-2M) using ammonium acetate. Preliminary characterization confirmed the nature of organo-Cr(III) complexes. Further characterization of these species by electrospray ionization mass spectrometry (ESI-MS) is in progress. Task 2. Demonstrate that chromate reduction produces organo-Cr(III) complexes with microbial cellular components. In the past year, further research on the formation of organo-Cr(III) complexes has been completed. Formation of soluble complexes with cell free extracts as the organic portion has resulted in the formation of organo-Cr(III) complexes, approximately 27% Cr(III) remained soluble after 14 days. In addition, complexes formed between individual organic components and Cr(III) have been tested for changes in solubility due to changes in pH. Results demonstrate that the samples remain soluble over the pH range typically encountered in the environment. These results have been reported in *Environmental Science and Technology* (2005, 39:2811-2817). Further work is targeted at structurally characterizing the organo-Cr(III) complexes. In order to characterize organo-Cr(III) complexes, we have synthesized five Cr(III) compounds with the organic ligands of acetate (ac), oxalate (ox), gluconate (glu), ethylenediamine (en), acetylacetonate (acac) and diethyldithiocarbamate (ddtc). These compounds represent a variety of electronic configurations, coordination environments, and donor atoms, e.g. O, N, and S donors. The electronic configurations, coordination environments, and donor atoms chosen are intended to represent common possibilities for bioreduced chromate. They also give us a wide range of possible Cr(III) coordination environments to study. Our characterization schemes represent two different approaches. One is to use mass spectrometry (MS) and extended X-ray absorbance fine structure (EXAFS) to identify structural characteristics of the various organo-Cr(III) species. In some cases, capillary electrophoresis (CE) is coupled with mass spectrometry (MS) through an electrospray ionization (ESI) interface. The CE allows us to use separations techniques to isolate distinct species when multiple species are present. Our second approach is to study electronic configurations using various advanced analytical techniques, including UV-visible absorbance spectroscopy and electron paramagnetic resonance spectroscopy (EPR). Information on electronic configurations is often necessary to fully

understand structural information. An example of this is the elucidation of two separate Cr(III) centers in the chromodulin protein in human systems. For our work, the Cr(III)-gluconate system is an example of how we can combine the information from a variety of techniques to understand the nature of the Cr(III)-glu organo complex. We prepared samples at pH 3.0 and gluconate to Cr(III) ratio around 3.0. We characterized the Cr(III)-organo complexes using CE-ESI-MS. We also used our Cr(en)<sub>3</sub>Cl<sub>3</sub> and Cr(acac)<sub>3</sub> model compounds as standards. We have identified two main mononuclear gluconate complexes with positive charges. Those two complexes were then isolated and obtained with Sephadex cation exchange resin. The EPR spectra of those Cr(III)/gluconate complexes indicate they have two different spin characters, i.e., one is high spin and the other low spin. We are now planning to further characterize these two complexes with EXAFS to obtain direct structure information. We will continue this study by incrementally increasing pH to the neutral range. These procedures and accumulated experience will be applied to other ligand, e.g., Cr(III)-ascorbate, Cr(III)-NAD<sup>+</sup>, etc. Task 3. Investigate the biological and abiotic transformation of organo-Cr(III) complexes formed during microbial reduction of chromate. Continued investigation into the biological transformation of the organo-Cr(III) complexes has resulted in the isolation of 13 additional bacteria capable of mineralizing the NAD<sup>+</sup>-Cr(III) complexes for growth. One of the new bacteria was isolated from a field sample taken from the Hanford site. The new isolates are all capable of using the NAD<sup>+</sup> ligand as the carbon and energy source, resulting in the precipitation of Cr(III) from solution, as demonstrated by 5 selected isolates (data not shown). All the isolates grow very slowly on NAD<sup>+</sup>-Cr(III). The bacteria have been classified based on their 16S rRNA gene sequences and assigned to one of the following genus, *Pantoea* (G-), *Leifsonia* (G+), *Rhodococcus* (G+), *Microbacterium* (G+), or *Pseudomonas* (G-). Thus, both Gram positive and Gram negative bacteria can mineralize organo-Cr(III). Further investigations will be aimed at 1) electron microscopy studied of precipitated Cr(III) on the bacterial surface, 2) isolation of bacteria capable of mineralizing other organo-Cr(III) complexes, e.g. ascorbate-Cr(III) or citrate-Cr(III), and 3) abiotic transformation of the organo-Cr(III) complexes. Task 4. Transport and fate of organic-Cr(III) complexes in soil. Batch equilibrium soil sorption experiments using synthetic organo-Cr(III) complexes (Serine-Cr(III), cysteine-Cr(III) and malate-Cr(III)) were not successful due to gradual soil attrition during agitation, which resulted in an increase in sorption surface area and capacity over time. Therefore, we conducted soil sorption experiments using continuous flow soil columns. The soil used was a silty sand from the Hanford Reservation in Washington State, which has an organic carbon content of 0.22%, a median particle size of about 0.1 mm, and a cation exchange capacity of 9.1 mol(+)/Kg. The experiment for malate-Cr(III) has been completed (Data not shown). The inert tracer (bromide) profile represents non-reactive soil column performance. The shift to the right for the malate-Cr(III) complex profile indicates a modest level of retardation, indicating that the malate-Cr(III) complex sorbs to the soil. The continuous flow column experiments with other complexes are in progress. These experiments will be followed with soil sorption studies of the purified compounds obtained directly from bacterial cultures.

**DELIVERABLES:** One publication and two manuscripts in preparation:

Puzon, G. J., Roberts, A. G, Kramer, D. M, and Xun, L. 2005. Formation of soluble organo-chromium(III) complexes after chromate reduction in the presence of cellular organics. *Environ. Sci. Technol.* 39:2811-2817.

Puzon, G. J., Tompkins, K. A., and Xun, L. Mineralization of a soluble organo-chromium(III) complex by novel bacterial isolates. In preparation.

Tokala, R., Peyton, B. et al. Identification, isolation and characterization of soluble organo-Cr(III) species produced by different bacteria. In preparation.